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Review

Characterization, structure and function of linker polypeptides in phycobilisomes of cyanobacteria and red algae: An overview

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Abstract

Cyanobacteria and red algae have intricate light-harvesting systems comprised of phycobilisomes that are attached to the outer side of the thylakoid membrane. The phycobilisomes absorb light in the wavelength range of 500–650 nm and transfer energy to the chlorophyll for photosynthesis. Phycobilisomes, which biochemically consist of phycobiliproteins and linker polypeptides, are particularly wonderful subjects for the detailed analysis of structure and function due to their spectral properties and their various components affected by growth conditions. The linker polypeptides are believed to mediate both the assembly of phycobiliproteins into the highly ordered arrays in the phycobilisomes and the interactions between the phycobilisomes and the thylakoid membrane. Functionally, they have been reported to improve energy migration by regulating the spectral characteristics of colored phycobiliproteins. In this review, the progress regarding linker polypeptides research, including separation approaches, structures and interactions with phycobiliproteins, as well as their functions in the phycobilisomes, is presented. In addition, some problems with previous work on linkers are also discussed.

Keywords: Phycobilisome; Linker polypeptide; Phycobiliprotein; Separation; Structure; Interaction; Function; γ Subunit

1. Introduction

The process of photosynthesis is initiated by the absorption of light. Cyanobacteria and red algae use multimeric protein complexes, the phycobilisomes (PBSs), as their major light-harvesting complexes (LHCs) [1,2]. PBSs are multimolecular structures made up of several polypeptide species that are biochemically separated into two types (Fig. 1). Phycobiliproteins (PBPs), primarily composed of α, β polypeptides (in some phycoerythrins, there is a special type of subunit, the γ subunit), are a brilliantly colored group of disc-shaped proteins bearing covalently attached open-chain tetrapyrroles known as phycobilins and are the main components of, and orderly assembled into, the PBSs [3]. In view of their spectral properties as well as pigment compositions, PBPs were divided into four groups, allophycocyanin (APC), phycocyanin (PC), phycoerythrin (PE) and phycoerythrocyanin (PEC). In addition, associated with the PBPs in these complexes are small amounts of linker polypeptides, most of which do not bear chromophores [4–6].

In an examination of the PBSs from eight species of cyanobacteria, Tandeau de Marsac and Cohen-Bazire [7] firstly demonstrated the presence of several colorless polypeptides in the PBSs by SDS-PAGE. Since this time, it had been estimated that as much as 12–15% of the total stainable proteins in the PBS components is accounted for by linker polypeptides [8]. They function to stabilize the

Abbreviations: LHC, light-harvesting complexes; PBS, phycobilisome; APC, allophycocyanin; PC, phycocyanin; PE, phycoerythrin; PEC, phycoerythrocyanin; PBP, phycobiliprotein; SDS-PAGE, sodium dodecyl sulfate-polyacrylamide gel electrophoresis; PEB, phycoerythrobilin; PUB, phycourobilin

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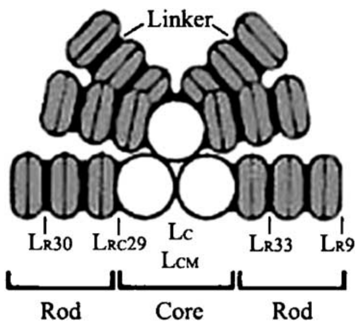

Fig. 1. Structural model of the hemidiscoidal phycobilisome [2]. The three white circles represent the tricylindrical core that is surrounded by an arrangement of six rods. Each rod is composed of a certain number of double-disc-shaped phycobiliproteins, shown in grey. The linkers are represented by black discs, and the related symbols are located in the central hole of the hexamers.

PBS structure and optimize the absorbance characteristics and energy transfer of the PBPs to favor a unidirectional flow of excitation energy from the peripheral PBPs of the rod to the PBS core, and then from the core to the photosynthetic reaction center [9].

The structures and functions of the PBPs have been extensively studied for decades, and a series of reviews concerning these findings have been published [1,2,4,5,9–14]. To date, however, less systematic work concerning linker polypeptides has been done, and few special and comprehensive reviews on this subject are available. The present review predominantly focuses on the structures and functions of linker polypeptides in the PBS, as well as the recent findings in linker polypeptide research. The goal of this review is to elucidate linker polypeptide characteristics and their corresponding biological functions in energy transfer within the PBS.

2. Classification

Glazer [10] has provided a system of abbreviations to characterize linker peptides. This widely-used classification system defines linker polypeptides with respect to their locations and molecular masses. L_X^Y refers to a linker polypeptide (L) having an apparent mass of *Y* kDa, located at position *X* in the PBS, where *X* can be R (rod), C (core), RC (rod–core junction) or CM (core–membrane junction) (Table 1) [15,16].

Using this classification system, linker polypeptides can be divided into four groups according to their functions and locations in the PBS: Group I, L_R polypeptides, having molecular weights of 27 to 35 kDa (involving small rod linkers of 10 kDa), participates in the assembly of the peripheral rod substructure. Polypeptides in this group are involved in linking PE/PC trimers to hexamers or PE/PC hexamers to other PE/PC hexamers; Group II, L_{RC} polypeptides, designated as having molecular weights of 25 to 27 kDa, is involved in attaching the peripheral rods to the APC cores and may function in the assembly of rod substructure; Group III, L_C polypeptides, with smaller molecular weights, is a portion of core components and plays key roles in the assembly of the core substructure; and Group IV, L_{CM}, which has molecular weights varying from 70 to 120 kDa depending on the organism from which they are isolated, is involved in the attachment of the PBSs to the membrane and is envisaged as one type of the terminal acceptors of excitation energy within the PBSs [11,17].

3. Separation

Previous studies on linker polypeptides have predominately focused on the PBP–linker complexes [16,18,19], and research on pure linker polypeptides is not commonly available due to the low yield and unique qualities of linkers. In addition, linker polypeptides are highly susceptible to proteolytic degradation, even in the presence of proteinase inhibitors [20]. Thus, the development of effective and rapid techniques to isolate linker polypeptides from the PBS is required.

The SDS-PAGE system is universally used to separate PBS components. In the stained gel, it is very easy to distinguish the linkers and PBP subunits because of their vastly different molecular weights, as well as their relative

Table 1
Characteristics of the major linker polypeptides

Protein	Symbol	Amino acid	MW (kDa)	p*I*	Annotation	Gene
CpeC	PE L_R	285–294	31.8–33.1	9.6	PE-associated linker	cpeC
CpeD	PE L_R	249–255	27.9–28.4	8.2–8.6	PE-associated linker	cpeD
CpeE	PE L_R	244–254	27.1–28.4	9.7	PE-associated linker	cpeE
PecC	PEC L_R	278–279	31.3–31.5	9.6–9.7	PEC-associated linker	pecC
CpcC	PC L_R	219–291	24.8–32.6	9.5–9.6	PC-associated linker	cpcC
CpcD	PC L_R	70–87	7.8–9.9	9.8–10.5	Rod capping linker	cpcD
CpcG	L_{RC}	231–279	26.8–31.9	9.3–9.6	Rod–core linker	cpcG
CpcH	L_{RC}	271–273	30.4–30.8	8.8–9.7	Rod–core linker	cpcH
CpcI	L_{RC}	288	32.7	8.9	Rod–core linker	cpcI
ApcC	L_C	66–69	7.7–7.8	10.9–11.4	APC–associated linker	apcC
ApcE	L_{CM}	683–1155	76.5–129.8	9.5–9.7	Core–membrane linker	apcE

The MW and p*I* values of each type of linker polypeptides were analysed by Antheprot v5.0.

amount in the entirety of PBS constituents. L_{CM} has previously been purified by SDS-PAGE and then transferred to a Trans Blot membrane [21,22]. However, future studies on the structure and function of linker polypeptides will require more effective approaches that will allow for their study in a solution in the absence of PBP.

The isolation of intact PBS from cyanobacteria and red algae allows for the detection of linker polypeptides. The common principle of the isolation of linker polypeptides from intact PBSs is their basicity (pI > 7.0) as compared to the relatively acidic (pI < 5.0) PBPs [23]. Interestingly, previous studies showed that the linkers dissolved in 8 M urea precipitated during the dialysis against 10 mM acetic acid, which implied that they were insoluble in aqueous buffers [24]. Moreover, in the absence of denaturing agents, the colorless polypeptides remained associated with the PBPs. Therefore, it is necessary to utilize a strong denaturant (8 M urea or 6 M guanidine) during the entire purification process in order to dissociate the PBS completely and to keep the linker polypeptides in a soluble state [23]. By using this approach, the 30- and 33-kDa linker polypeptides have been purified from *Synechococcus* PCC 6301 [24]. This approach was also effective in the separation of the γ subunit, a colored polypeptide which is generally considered a specific linker polypeptide in red algae PE [25] (the details of the γ subunit are discussed below). After treatment with 8 M urea for 12 h, R-PE from *Corallina officinalis* was injected on a CM-Sepharose FF column, and the γ subunits, with a mass of 31 kDa, were collected.

As an alternative method, HPLC has been successful in analytically separating linker polypeptides from intact PBSs due to the different hydrophobic properties of linkers. Four colorless linkers, identified as L_C^{8}, L_R^{10}, L_R^{33} and L_{RC}^{29}, were obtained in the separation of the PBS components of *Spirulina maximum* and *Synechocystis* PCC 6803 by C_4 reversed-phase chromatography [26], and the subsequent identification of each component was monitored by MS [27,28]. This methodology is powerful, simple and precise in detecting the differences in the protein constituents of PBS. Similarly, the qualitative differences between γ subunits and α, β polypeptides (see Section 4.2) in PE also allow for the effective separation of γ subunits by HPLC [29–31].

The PBS substructures, which are simpler than intact PBSs in composition, are often regarded as the ideal samples in the separation of linkers. Füglistaller and his coworkers [19] isolated $L_R^{34.5}$ (PEC), $L_R^{34.5}$ (PC) and a linker polypeptide, $L_C^{8.9}$, from their respective PBP complexes by gel filtration on a Bio-Gel P-100 column in 50% formic acid. Similarly, a preparative isolation of the $L_C^{8.9}$ from $(\alpha\beta)_3^{APC}$ of *Mastigocladus laminosus* by reverse phase chromatography has also been presented [20].

In order to effectively isolate low-content linker polypeptides, gene-engineering technologies have been developed to produce linker polypeptides in the PBSs. A T7 RNA polymerase expression system was used to express $L_C^{8.9}$ from *M. laminosus* in *Escherichia coli*, and the products were expressed as inclusion bodies that were stabilized in a buffer containing 8 M urea [20]. These results reported that $L_C^{8.9}$ overexpressed in *E. coli* retained the biological function of reconstituting the complex $(\alpha\beta)_3{}^{APC}L_C^{8.9}$. Therefore, molecular techniques can also serve as effective tools in large-scale preparation of linker polypeptides.

4. Structure and interaction with phycobiliproteins

4.1. Primary structure

A number of the amino acid sequences of linker polypeptides have been determined by deducing from the nucleotide sequences or via direct sequencing of the polypeptides. The sequences indicate that linker polypeptides are generally basic proteins with pI of 8–9. In addition, the alignment results indicate that the primary sequences of linkers are less conserved than those of their associated PBP subunits, which typically exhibit approximately 75% sequence homology among different types of PBPs. In the core of PBS, L_C shows a comparatively high identity among linker polypeptides from different origins of algae, indicating conservation among ancestral linker polypeptides. Likewise, in the rod linker polypeptides, many conserved structural motifs have been reported. Up to six conserved domains near the N-terminus are presumed to play crucial roles in packing into the central channels of the PBP hexamers, whereas other regions may provide the assembly interface between rod disks [4,24,32,33]. The rod–core linkers are more distantly related to the rod linker polypeptides. However, six conserved domains have also been identified within the N-terminal of these linker proteins [34], which were presumed to occupy the central hole of $(\alpha\beta)_3^{PC}$ because the 22-kDa fragment is largely protected in proteolytic treatments. Considering the less conserved C-terminal of L_{RC} in contrast to that of L_R, it is believed that L_{RC} may specifically connect with distinct regions of the core complexes. Based on the sequence analysis and proteolysis experiments of linker polypeptides, Parbel et al. [6] proposed an interlocking model for the PBS rod organization, in which the linker polypeptides in the model are proposed to have two distinctive domains (Fig. 2): The N-terminal domain is hypothesized to be buried in the central hole of the trimer and protected from proteolysis, whereas C-terminal domain is believed to protrude from the hexamer.

The apcE gene product, L_{CM}, otherwise known as the anchor polypeptide, is the largest component of the PBS [24,35]. Corresponding to the bicylindrical, tricylindrical or pentacylindrial types of cores in different algae, the molecular masses of L_{CM} can be classified into three types, 70,000 to 75,000, 92,000 to 99,000 and 115,000 to 128,000, respectively [36]. In the core complex, L_{CM} has

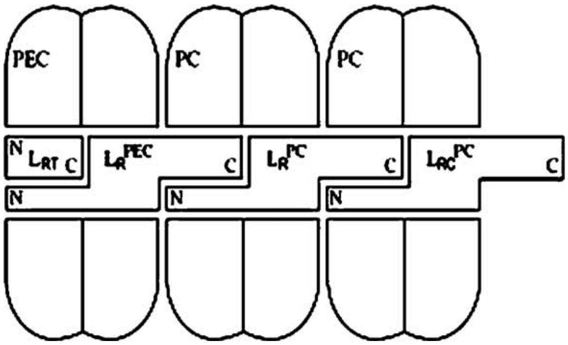

Fig. 2. Model of the interlocking linker polypeptides from *M. laminosus* [6] (shown for a rod containing one phycoerythrocyanin and two phycocyanin hexameric disks). The linkers are generalized as L_{RT} (rod terminating linker), L_R^{PEC}, L_R^{PC} and L_{RC}, respectively. "N" represents the N-terminus and "C" means the C-terminus of linker polypeptides. It is proposed that linkers protrude from one phycobilisome disk into the neighbouring one.

multiple large domains with similarity to rod linker sequences that may support APC hexamer formation (Fig. 3) [37,38]. Regarding the immunological properties [39] and the structural model of L_{CM}, it is commonly believed to be a special linker polypeptide. Sequence analysis has defined several domains within the L_{CM}, including an N-terminal PBP-like domain followed by several L_R^{PC}-like domains. The N-terminal domain (PB domain) with a cysteine-binding site for a single PCB (e.g. Cys186 of *Synechococcus* sp. PCC 7002) is homologous to the amino acid sequences of the PBP subunits. A small insertion fragment following the PB domain forms a loop domain (PB loop) that is believed to protrude from the trimer and play a key role in the PBS anchoring to the thylakoid membrane surface [40]. However, this function is not supported by previous findings which showed that this domain of the L_{CM} seems dispensable for PBS biogenesis and function [41]. The C-terminal portion of the L_{CM} includes some repeat domains (REP domains), which exhibited a high degree of homology to L_R. These domains are likely to be involved in the interactions with APC and the assembly of the PBS core. Furthermore, the arm domains (ARM domains), which are located between PBP and REP domains, may form the connections between different PBS cores.

4.2. Advanced structure and interactions with phycobiliproteins

In the past few decades, the highly efficient energy transfer from the light-harvesting antenna PBS to the reaction center, as well as the physiological principles of the PBPs in vivo, have been elucidated through structure analysis of X-ray data derived from the PBP crystals [42–49]. The PBP trimer, which is composed of three α and three β subunits, forms a flattened disk ($\sim$11 nm diameter) with a central cavity. The hexameric PBP complexes are comprised of two trimers arranged "face to face" with an internal cavity. It is generally believed that the cavity, with a diameter of approximately 3.5 nm, is occupied by linker polypeptides (Fig. 4). This hypothesis was supported by our findings regarding the structure of C-phycocyanin in *Spirulina platensis* with STM [50,51].

In contrast to the widely studied and well-reported structures of the PBPs, the 3D structure of linker polypeptides has only been provided in an APC–linker complex including linker polypeptide $L_C^{7.8}$ [52]. The PBPs with and without linkers crystallized primarily in trigonal or hexagonal space groups occupying a threefold axis of symmetry,

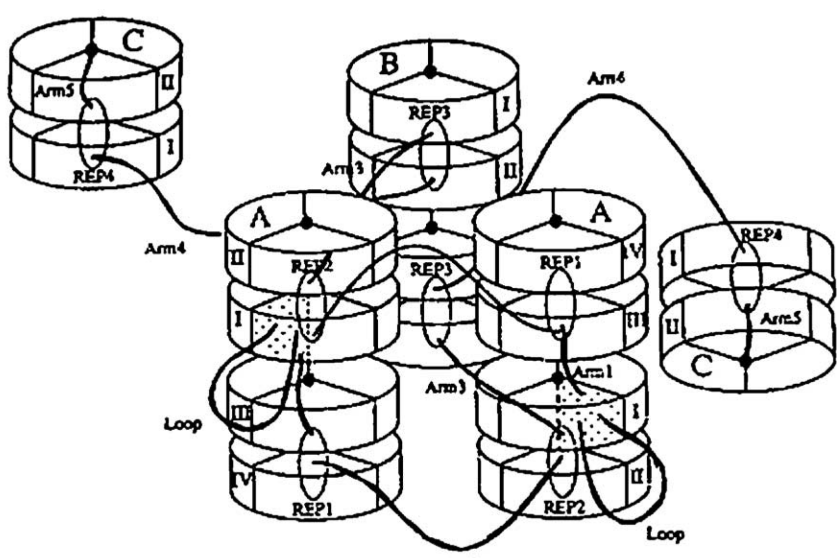

Fig. 3. Model of the structure of core–membrane linker L_{CM} [37]. Three distinctive domains in the model of L_{CM} are designated as Arm, REP and Loop domains. The letters A and B represent the two basal cylinders and the top cylinder of APC, respectively. The letter C represents the additional APC-hexamers. I and II represent the two trimers of APC. The ARM and the REP domains, which play the connecting roles in PBS core assembly, are symbolized as the Arm and REP, respectively. There are still two loop domains of L_{CM} having unclear functions at present.

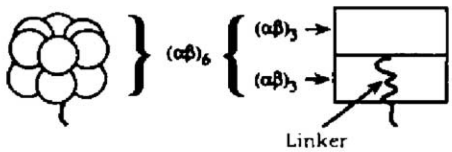

Fig. 4. Model of the orientation of linker polypeptides in phycobiliprotein hexamers [33]. According to the dissociation and proteolysis experiments, the linkers have two distinctive domains. The large domain buries in the central hole of the trimer, while the small domain protrudes from the hexamer. In this model, the linker interacts with only one of the trimers instead of, in Fig. 2, with both trimers. This difference presents an uncertain conclusion on the interacting sites between linkers and phycobiliproteins.

thus leading to a statistical disorder of the linker moiety [53]. Thus, it was deduced that the linker polypeptide disrupted the C_3 symmetry in the central cavity of the associated PBP, and this asymmetric interaction could serve to guide the transfer of excitation energy along the PBS rods toward the core elements [16].

The structural data from the APC–linker complex indicates that this linker polypeptide has an elongated shape and consists of a three-stranded β-sheet (β1, Leu-3-Leu-9; β2, Tyr-26-Pro-32; β3, Lys-49-Leu-55), two α-helices (α1, Leu-22-Thr-25; α2, Tyr-33-Met-46) and the connecting random coil segments (Fig. 5). The $L_c^{7.8}$ is in contact with only two of the three β subunits and binds via multiple charged, polar and hydrophobic interactions to the protein chains of these monomers [54], thereby bringing the β84 chromophores closer together and optimizing the directed energy transfer within the trimer and between the constitutes of the core [55].

The protein surfaces of linker polypeptides are not similar to those of globular proteins in that they are most likely hydrophobic. Together with the mentioned finding that they are positively charged and the PBP are negatively charged, it suggests that linker polypeptides and the PBPs interact by a combination of hydrophobic and multiple charged interactions [4,56,57]. The observation of the sequence homology between different linker polypeptides and between their associated β subunits of PBPs (buried in the central cavity of the PBP hexamers), as well as their conserved basic and acidic amino acids, is better explained under the assumption that these domains are sites of PBP–hexamer/rod and rod–core linker interactions [34]. Highly conserved residues that are unique to the individual subunits are involved in the interactions between PBPs and linker polypeptides [2]. The six domains of linkers with high homology mentioned previously are likely to be the sites of structural interactions close to the corresponding PBPs.

Recently, new bioinformatical methods, such as sequence alignments and molecular simulations, as well as reconstitution experiments, have allowed for future research on the interactions between linker polypeptides and the PBPs.

5. Function

The contribution of each linker polypeptide to the function of an intact PBS has been gradually inferred from findings that the composition of the PBS is altered in vivo by mutation [58,59], in vitro reconstitution experiments or by external perturbations, such as nutrient and sulfur limitation or changes to the intensity or wavelengths of illumination for cell growth [32,60,61].

Linker polypeptides are thought to play two roles in the PBS: (1) provide structural connections between adjacent PBPs and stabilize the PBS structures, and (2) modulate the absorption and fluorescence properties to facilitate or directly participate in energy transfer from the rod to the core and eventually to the chlorophyll-containing thylakoid membrane of the photosynthetic cells. The assembly of the PBS is mediated by linker polypeptides, and each of the trimeric or hexameric subassemblies of the PBS contains at least one specific linker polypeptide, which determines the type, location and aggregation state of the PBP within the rod and also modulates the spectroscopic properties [14].

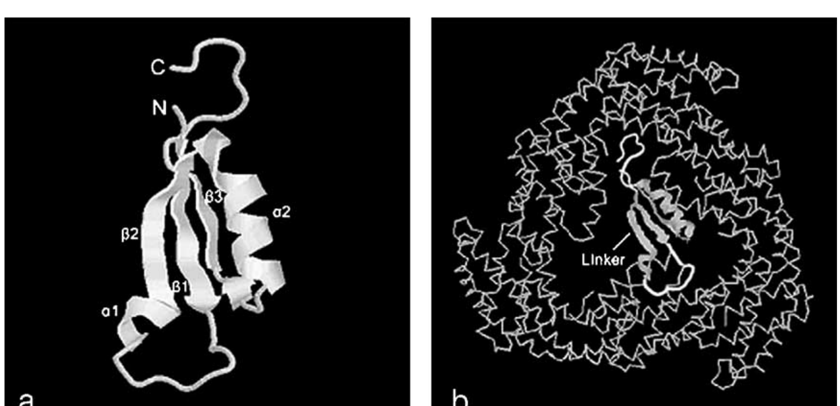

Fig. 5. Structure of the entire APC-$L_C^{7.8}$ complex from *M. laminosus* [54]. (a) Tertiary structure of $L_C^{7.8}$ in the phycobilisome from *M. laminosus.* The secondary structural elements of the linker polypeptide are represented as ribbons. Three-stranded β-sheets, two α-helices and the connecting random coil segments are shown. (b) The $L_C^{7.8}$ is buried into the central hole surrounded by subunits of allophycocyanin in sticks.

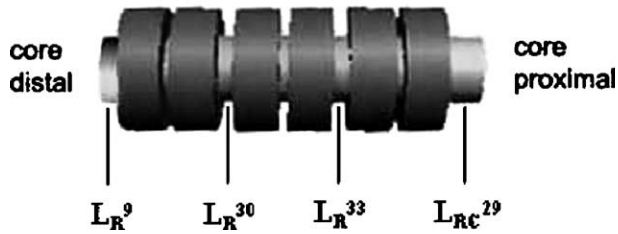

Fig. 6. Model of the phycobilisome rod complex from *S. platensis*. Specific linkers have distinct orientations and combine with different phycobiliproteins in the rod.

The position of a hexamer within the rod structure appears to be governed by the specific linker polypeptide with which it is associated (Fig. 6). The core-proximal rod disc contains the linker with the lowest mass (27,000 to 29,000); the next disc has the linker with the greatest mass (32,500 to 33,500), and the third disc contains a linker of intermediate mass (29,000 to 30,000) [62]. Different linker polypeptides at distinct sites contribute to different functions of the PBS. For example, the small PC rod linker polypeptide is thought to limit the number of hexamers assembled in a PBS rod by preventing the addition of other hexamers. Increasing proportions of $L_R^{8.2}$ in *Synechococcus* 6301 inhibited the elongation of rods, and therefore, it has been named rod-terminating linker [17]. In vitro assembly studies indicate that 30- and 33-kDa linker polypeptides from the same cyanobacteria could stimulate long rod growth, whereas the 27-kDa polypeptide could nucleate the formation of discs and terminate rod assembly [24]. This finding was supported by the report that the 29-kDa polypeptide from the *Nostoc* sp. was a crucial factor for reconstituting PBS and that its role in maintaining PBS structure was most likely to attach the PE-PC-containing rods to the core [63]. It was also shown that L_R^{33} from *M. laminosus* and *Synechococcus* sp. PCC 7002 is involved in the attachment of the core-distal PC hexamer to the core-proximal one, and L_{RC} connects the PC hexamer of rods to specific domains of the core. $L_C^{8.9}$ does not appear to be essential for PBS assembly, but is essential for improving the stability and energy transfer of PBS [59,64]. The sequence similarity of $L_C^{7.7}$ to L_R^9 suggested that it might perform a similar capping function in short rods which make up the core. In addition to the key role of the linkers, Zhang et al. [65] found that the in vivo assembly of PBPs is also dependent on the endogenous properties of PBP themselves and that purified R-PE molecules could self-assemble "disc to disc". These findings supported the idea that linker polypeptides have a dual function in forming PBS structure: stabilization and localization.

Complementary chromatic adaptation (CCA) resulted in a serials of changes to the PBS composition and structure [66–68]. In strains undergoing chromatic adaptation, the termination of PE synthesis was accompanied by a decrease in the intensity of one linker-related band in SDS-PAGE gel, whereas with increased PC synthesis, two other bands increased [15]. High light brings on a decrease in the specific 33-kDa linker contents [69], which supports the presence of a specific linker with each disk at a particular rod location.

In addition to the general coordination and stabilization effects, the influence of linkers is indicated by the spectral changes of the associated PBPs, as well as the alternations of energy transfer in the PBPs. The isolation of a PBP with and without an associated linker protein provides a good system in which to study the roles of linkers in modifying the energy transfer of the PBPs complexes [16,70]. In the APC–$L_C^{7.8}$ complex, $L_C^{7.8}$ leads to an absorbance shift to 653 nm and a decreased shoulder near 620 nm [54]. Similarly, $L_C^{8.9}$ from *M. laminosus* does not influence the α^{APC} or the $\alpha^{AP\text{-}B}$ chromophores, but it shifts the absorbance maximum of the β^{APC} chromophore to a longer wavelength than that of the α^{APC} chromophore in trimeric complexes. L_{RC} occupies the key position in the PBS structure and energy transfer pathway. Its function has been demonstrated in vivo by mutational studies in which CpcG was inactivated. It was found that L_{RC} from *M. laminosus* caused a red shift of 15 nm when associated with a PC trimer [71]. The homologous red shift in the spectral absorption maximum and a modification of the chromatographic properties of the PC induced by 27-, 30- and 33-kDa linker polypeptides has also been reported [24]. Trimeric or hexameric complexes of the PC with different linker polypeptides display distinct absorption, fluorescence and circular dichroism spectra [12]. The absorption and fluorescence emission spectra of the $(\alpha\beta)_6^{PC}L_{RC}^{27}$ complex are shifted red relative to those of $(\alpha\beta)_6^{PC}L_R^{33.5}$, indicating that the influence of L_{RC} on the red shift in the absorbance and fluorescence emission is more potent than that of L_R [18]. As a result, the favored direction of transfer is from the distal disc to the PC disc proximal to the core. The sum of these linker-induced effects, including PBP modulation and distinct spectroscopic properties, as well as the attraction of the chromophores, optimizes the directional energy transfer within the molecule from one acceptor bilin to the one below it toward the core [14,24].

6. γ Subunit—a special linker polypeptide in the phycoerythrin

In PEs of red algae, there are special polypeptides, designed γ subunits, which are structurally different from the α and β subunits. These subunits link PE hexamers to the rod subcomplexes, playing a role similar to that of linkers in cyanobacterial PBSs. Because of this, they are thought as a type of rod linker polypeptides with molecular weights of approximately 30 kDa [72], serving as PE linker polypeptides. In contrast to other linkers, however, they are chromophorylated, containing two types of covalently attached linear tetrapyrrole chromophores, phycoerythrobilin (PEB) and phycourobilin (PUB).

The γ subunits are nuclear encoded, whereas linkers in red algae are plastid encoded [72]. Of the available sequence data, no high degree of sequence homology was found between the γ subunits and other linker polypeptides. It may be the case that there is a range of balanced states for different primary structures, which still allows for similar physiological functions.

It is well known that PE can be classified into four classes: C-PE, b-PE, B-PE and R-PE, based on their origins and absorption properties [73,74]. Pan et al. have researched the spectral properties of PEs in marine red algae and have reported that R-PEs have two spectral types, "two-peak" type and "three-peak" type. These results suggest that the diversity of PE may be related to the properties of their γ subunits [30,75]. One possibility is that geometrical variations of chromophores depend on the linker association. Due to their similarity to cyanobacterial linkers, γ subunits are also possible to fill the central hole of a single hexamer [76], and their high p*I* values (8–9) stimulate tight interactions with PE hexamers. It has therefore been suggested that the conserved arginine and glutamic acid residues of γ subunits are necessary for their interactions with the PE subunits [77] and that the two tyrosine residues of the γ subunits may be the sites of interaction with PE hexamers [76]. Wilbanks and Glazer [78] hypothesized that in a PE hexamer, only the N-terminal portion of the γ-subunit occupies the central hole, whereas the cleavable C-terminal domain protrudes from the central hole.

Except for the function of linking and stabilizing rod substructures, like linkers in cyanobacteria, the γ subunits carry PEB and PUB, which facilitate PE hexamers' ability to effectively participate in light-harvesting and energy transfer. Regarding the optical properties, the 550-nm absorption is due to the presence of PEB and the 495 nm is due to the presence of PUB. Interestingly, the PUB chromophores shift the absorption range toward the shorter wavelengths; a large number of chromophores benefit the PBS due to the tendency for the subunits and linkers to attach as many bilins as possible during the process of evolution [1].

7. Perspectives

The discovery of colorless polypeptides in the PBS has generated great interest and speculation regarding the possible function of these polypeptides. Recently, the techniques of biochemistry, molecular biology, spectroscopy, X-ray diffraction, electron microscopy and biophysics have served to improve our understanding of how these proteins perform their functions in photosynthesis [13,65,79]. The isolation and characterization of specific PBS components from red algae should also contribute to our knowledge about the localization of the encoding sites for different PBS components, as well as the regulation of their synthesis.

The importance of linker polypeptides for the assembly of defined complexes and their roles in the tuning of the spectral characteristics of the complexes are well accepted. Unfortunately, at present, our understanding of linkers is minimal compared to our understanding of the PBPs, and many aspects of linker polypeptide activity remain to be elucidated:

(1) It is essential to develop effective separation methods in order to obtain active linkers from the PBS instead of previous methods in denatured conditions. The expression of linkers in *E. coli* combined with the chromophorylation of colored linkers [80] has exhibited a great field for future functional studies of linker polypeptides. (2) The native tertiary structures of linker polypeptides are required in order to understand the entire energy flow pathway. (3) What are the accurate influences of bilins near the central hole on the spectra and energy transfer kinetics? (4) How do linkers perform the specific connections to the corresponding PBPs or other linkers? (5) Previous work has predominately focused on the linker polypeptides of some model strain cyanobacteria (e.g. *Synechococcus*, *Spirulina* and *Mastigocladus*) and unicellular red algae (e.g. *Porphyridium*). The observations of linker polypeptides from higher multicellular and marine macroalgae are comparatively scarce. (6) The positions of highly conserved PBPs are determined by the specific linker polypeptides, which are exhibited in the varieties of PBSs. Therefore, the evolution of linker polypeptides is also an important aspect of understanding the function of the PBS. In particular, the γ subunit in red algae and marine cyanobacteria attracts a great deal of attention, and its relationship with other linker polypeptides is significant to the study of PBS evolution. (7) A number of questions concerning the γ subunits in red algae remain unanswered, such as the interactions with PE hexamers and the evolutionary origins of these subunits. Our ability to answer such questions relies on our ability to develop relevant biochemical, structural and bioinformatical techniques.

The sum of the current work on linker polypeptides will facilitate our understanding of their biological functions and the complicated interactions between linker polypeptides and the PBPs. Future research will drive the field towards a greater understanding of the evolutional mechanisms of photosynthetic LHCs in cyanobacteria and red algae.

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References

[1] L. Talarico, G. Maranzana, Light and adaptive responses in red macroalgae: an overview, J. Photochem. Photobiol., B Biol. 56 (2000) 1–11.

[2] A.R. Grossman, D. Bhaya, K.E. Apt, D.M. Kehoe, Light-harvesting complexes in oxygenic photosynthesis: diversity, control, and evolution, Annu. Rev. Genet. 29 (1995) 231–288.

[3] E. Mörschel, W. Wehrmeyer, K.P. Koller, Biliprotein assembly in the disc-shaped phycobilisomes of *Rhodella violacea*. Electron microscopical and biochemical analysis of B-phycoerythrin and B-phycoerythrin-C-phycocyanin aggregates, Eur. J. Cell Biol. 21 (1980) 319–327.

[4] W.A. Sidler, Phycobilisome and phycobiliprotein structures, in: D.A. Bryant (Ed.), Molecular Biology of the Cyanobacteria, Kluwer Academic Publishing, Dordrecht, The Netherlands, 1994, pp. 139–216.

[5] A.N. Glazer, Phycobilisomes: a macromolecular complex optimised for light energy transfer, Biochim. Biophys. Acta 768 (1984) 29–51.

[6] A. Parbel, H. Scheer, Model for the phycobilisome rod with interlocking disks based on domain-weighted linker–polypeptide sequence homologies of *Mastigocladus laminosus*, Int. J. Photoenergy 2 (2000) 31–40.

[7] N. Tandeau de Marsac, G. Cohen-Bazire, Molecular composition of cyanobacterial phycobilisomes, Proc. Natl. Acad. Sci. U. S. A. 74 (1977) 1635–1639.

[8] E. Gantt, Structure and function of phycobilisomes: light harvesting pigment complexes in red and blue-green algae, Int. Rev. Cytol. 66 (1980) 45–80.

[9] K.E. Apt, J.L. Collier, A.R. Grossman, Evolution of the phycobiliproteins, J. Mol. Biol. 248 (1995) 79–96.

[10] A.N. Glazer, Light harvesting by phycobilisomes, Annu. Rev. Biophys. Biophys. Chem. 14 (1985) 47–77.

[11] B.A. Zilinskas, L.J. Greenwald, Phycobilisome structure and function, Photosynth. Res. 10 (1986) 7–35.

[12] A.N. Glazer, Phycobilisomes: structure and dynamics, Annu. Rev. Microbiol. 36 (1982) 173–198.

[13] R. MacColl, Cyanobacterial phycobilisomes, J. Struct. Biol. 124 (1998) 311–334.

[14] A.N. Glazer, Light guides. Directional energy transfer in a photosynthetic antenna, J. Biol. Chem. 264 (1989) 1–4.

[15] M. Glauser, W.A. Sidler, K.W. Graham, D.A. Bryant, G. Frank, E. Wehrli, H. Zuber, Three C-phycoerythrin-associated linker polypeptides in the phycobilisome of green-light-grown *Calothrix* sp. PCC 7601 (cyanobacteria), FEBS Lett. 297 (1992) 19–23.

[16] S.A. Pizarro, K. Sauer, Spectroscopic study of the light-harvesting protein C-phycocyanin associated with colorless linker peptides, Photochem. Photobiol. 73 (2001) 556–563.

[17] R. de Lorimier, D.A. Bryant, S.E. Stevens Jr., Genetic analysis of a 9 kDa phycocyanin-associated linker polypeptide, Biochim. Biophys. Acta 1019 (1990) 29–41.

[18] M. Glauser, W. Sidler, H. Zuber, Isolation characterization and reconstitution of phycobiliprotein rod–core linker polypeptide complex from the phycobilisome of *Mastigocladus laminosus*, Photochem. Photobiol. 57 (1993) 344–351.

[19] P. Füglistaller, F. Suter, H. Zuber, Linker polypeptides of the phycobilisome from the cyanobacterium *Mastigocladus laminosus*: I. Isolation and characterization of phycobiliprotein–linker–polypeptide complexes, Biol. Chem. Hoppe-Seyler 367 (1986) 601–614.

[20] M. Betz, U. Ruegsegger, A.M. Esteban, W.A. Sidler, H. Zuber, Reconstitution of the core complex $(\alpha\beta)_3^{APC}L_c^{8.9}$ of the phycobilisome from *Mastigocladus laminosus* using the $L_c^{8.9}$ linker polypeptide overexpressed in *Escherichia coli*, Biol. Chem. Hoppe-Seyler 374 (1993) 435–443.

[21] T. Redlinger, E. Gantt, A Mr 95,000 polypeptide in *Porphyridium cruentum* phycobilisomes and thylakoids: possible function in linkage of phycobilisomes to thylakoids and in energy transfer, Proc. Natl. Acad. Sci. U. S. A. 79 (1982) 5542–5546.

[22] C. Gomez-Lojero, B. Pérez-Gómez, D.W. Krogmann, A. Peña-Diaz, The tricylindrical core of the phycobilisome of the cyanobacterium *Arthrospira* (*Spirulina*) *maxima*, Int. J. Biochem. Cell Biol. 29 (1997) 959–970.

[23] D.J. Lundell, A.N. Glazer, Linker polypeptides of cyanobacterial phycobilisomes, Fed. Proc. 39 (1980) 1676.

[24] D.J. Lundell, R.C. Williams, A.N. Glazer, Molecular architecture of a light-harvesting antenna. In vitro assembly of the rod substructures of *Synechococcus* 6301 phycobilisomes, J. Biol. Chem. 256 (1981) 3580–3592.

[25] S. Wang, F.D. Zhong, Z.J. Wu, Q.Y. Lin, L.H. Xie, Analysis on subunit composition of a new phycoerythrin, J. Fujian Agric. For. Univ. 33 (2004) 69–71.

[26] C. Gomez-Lojero, B. Pérez-Gómez, G. Prado-Flores, D.W. Krogmann, A. Carabez-Trejo, A. Peña-Diaz, The phycobilisomes of the cyanobacterium *Arthrospira* (*Spirulina*) *maxima*, Int. J. Biochem. Cell Biol. 29 (1997) 1191–1205.

[27] L. Zolla, M. Bianchetti, S. Rinalducci, Functional studies of the *Synechocystis* phycobilisomes organization by high performance liquid chromatography on line with a mass spectrometer, Eur. J. Biochem. 269 (2002) 1534–1542.

[28] L. Zolla, M. Bianchetti, High-performance liquid chromatography coupled on-line with electrospray ionization mass spectrometry for the simultaneous separation and identification of the *Synechocystis* PCC 6803 phycobilisome proteins, J. Chromatogr., A 912 (2001) 269–279.

[29] R. Bermejo, E.M. Talavera, J.M. Alvarez-Pez, Chromatographic purification and characterization of B-phycoerythrin from *Porphyridium cruentum*. Semipreparative high-performance liquid chromatographic separation and characterization of its subunits, J. Chromatogr., A 917 (2001) 135–145.

[30] I.N. Stadnichuk, A.V. Khokhlachev, Y.V. Tikhonova, Polypeptide γ-subunits of R-phycoerythrin, J. Photochem. Photobiol., B Biol. 18 (1993) 169–175.

[31] R.V. Swanson, A.N. Glazer, Separation of phycobiliprotein subunits by reverse-phase high-pressure liquid chromatography, Anal. Biochem. 188 (1990) 295–299.

[32] L.K. Anderson, C.M. Toole, A model for early events in the assembly pathway of cyanobacterial phycobilisomes, Mol. Microbiol. 30 (1998) 467–474.

[33] M.H. Yu, A.N. Glazer, Cyanobacterial phycobilisomes. Role of the linker polypeptides in the assembly of phycocyanin, J. Biol. Chem. 257 (1982) 3429–3433.

[34] M. Glauser, V.L. Stirewalt, D.A. Bryant, W. Sidler, H. Zuber, Structure of the genes encoding the rod–core linker polypeptides of *Mastigocladus laminosus* phycobilisomes and functional aspects of the phycobiliprotein/linker–polypeptide interactions, Eur. J. Biochem. 205 (1992) 927–937.

[35] K.H. Zhao, P. Su, S. Böhm, B. Song, M. Zhou, C. Bubenzer, H. Scheer, Reconstitution of phycobilisome core–membrane linker, L_{CM}, by autocatalytic chromophore binding to ApcE, Biochim. Biophys. Acta 1706 (2005) 81–87.

[36] R. MacColl, Allophycocyanin and energy transfer, Biochim. Biophys. Acta 1657 (2004) 73–81.

[37] V. Capuano, A.S. Braux, N. Tandeau de Marsac, J. Houmard, The "anchor polypeptide" of cyanobacterial phycobilisomes. Molecular characterization of the *Synechococcus* sp. PCC 6301 apcE gene, J. Biol. Chem. 266 (1991) 7239–7247.

[38] J. Houmard, V. Capuano, M.V. Colombano, T. Coursin, d.M. Tandeau, Molecular characterization of the terminal energy acceptor of

cyanobacterial phycobilisomes, Proc. Natl. Acad. Sci. U. S. A. 87 (1990) 2152–2156.
[39] B.A. Zilinskas, D.A. Howell, Comparative immunology conserved of the phycobilisome linker polypeptides, in: J. Biggins (Ed.), Progress in Photosynthesis Research, vol. II, Martinus-Nijhoff, Dordrecht, The Netherlands, 1987, pp. 161–164.
[40] D.A. Bryant, V.L. Stirewalt, M. Glauser, G. Frank, W. Sidler, H. Zuber, A small multigene family encodes the rod–core linker polypeptides of *Anabaena* sp. PCC 7120 phycobilisomes, Gene 107 (1991) 91–99.
[41] G. Ajlani, C. Vernotte, Deletion of the PB-loop in the L(CM) subunit does not affect phycobilisome assembly or energy transfer functions in the cyanobacterium *Synechocystis* sp. PCC 6714, Eur. J. Biochem. 257 (1998) 154–159.
[42] T. Jiang, J. Zhang, D. Liang, Structure and function of chromophores in R-Phycoerythrin at 1.9 Å resolution, Proteins 34 (1999) 224–231.
[43] J.Y. Liu, T. Jiang, J.P. Zhang, D.C. Liang, Crystal structure of allophycocyanin from red algae *Porphyra yezoensis* at 2.2-Å resolution, J. Biol. Chem. 274 (1999) 16945–16952.
[44] M. Betz, One century of protein crystallography: the phycobiliproteins, Biol. Chem. 378 (1997) 167–176.
[45] X.Q. Wang, L.N. Li, W.R. Chang, J.P. Zhang, L.L. Gui, B.J. Guo, D.C. Liang, Structure of C-phycocyanin from *Spirulina platensis* at 2.2 Å resolution: a novel monoclinic crystal form for phycobiliproteins in phycobilisomes, Acta Crystallogr., D Biol. Crystallogr. 57 (2001) 784–792.
[46] A.K. Padyana, V.B. Bhat, K.M. Madyastha, K.R. Rajashankar, S. Ramakumar, Crystal structure of a light-harvesting protein C-phycocyanin from *Spirulina platensis*, Biochem. Biophys. Res. Commun. 282 (2001) 893–898.
[47] T. Jiang, J.P. Zhang, W.R. Chang, D.C. Liang, Crystal structure of R-phycocyanin and possible energy transfer pathways in the phycobilisome, Biophys. J. 81 (2001) 1171–1179.
[48] R. Ficner, K. Lobeck, G. Schmidt, R. Huber, Isolation, crystallization, crystal structure analysis and refinement of B-phycoerythrin from the red alga *Porphyridium sordidum* at 2.2 Å resolution, J. Mol. Biol. 228 (1992) 935–950.
[49] C. Contreras-Martel, J. Martinez-Oyanedel, M. Bunster, P. Legrand, C. Piras, X. Vernede, J.C. Fontecilla-Camps, Crystallization and 2.2 Å resolution structure of R-phycoerythrin from *Gracilaria chilensis*: a case of perfect hemihedral twinning, Acta Crystallogr., D Biol. Crystallogr. 57 (2001) 52–60.
[50] Y.Z. Zhang, D.X. Shi, B.C. Zhou, C.K. Zeng, S.J. Pang, Study on the structure of C-phycocyanin in *Spirulina platensis* with scanning tunneling microscope, Acta Biochim. Biophys. Sin. 29 (1997) 521–525.
[51] Y.Z. Zhang, X.L. Chen, B.C. Zhou, D.X. Shi, S.J. Pang, A new model of phycobilisome in *Spirulina platensis*, Sci. China, Ser. C 42 (1999) 74–79.
[52] P.J. Bickel, K.J. Kechris, P.C. Spector, G.J. Wedemayer, A.N. Glazer, Finding important sites in protein sequences, Proc. Natl. Acad. Sci. U. S. A. 99 (2002) 14764–14771.
[53] R. Ficner, R. Huber, Refined crystal structure of phycoerythrin from *Porphyridium cruentum* at 0.23-nm resolution and localization of the gamma subunit, Eur. J. Biochem. 218 (1993) 103–106.
[54] W. Reuter, G. Wiegand, R. Huber, M.E. Than, Structural analysis at 2.2 Å of orthorhombic crystals presents the asymmetry of the allophycocyanin–linker complex, AP·$L_C^{7.8}$, from phycobilisomes of *Mastigocladus laminosus*, Proc. Natl. Acad. Sci. U. S. A. 96 (1999) 1363–1368.
[55] J.M. Steiner, J.A. Pompe, W. Löffelhardt, Characterization of apcC, the nuclear gene for the phycobilisome core linker polypeptide L(c)(7.8) from the glaucocystophyte alga *Cyanophora paradoxa*. Import of the precursor into isolated cyanelles and integration of the mature protein into intact phycobilisomes, Curr. Genet. 44 (2003) 132–137.
[56] P. Fuglistaller, R. Rumbeli, F. Suter, H. Zuber, Minor polypeptides from the phycobilisomes of the cyanobacterium, *Mastigocladus laminosus*. Isolation, characterization and amino acid sequences of a colorless 8.9 kD polypeptide and of a 16.2 kD phycobiliprotein, Hoppe-Seyler Z. Physiol. Chem. 365 (1984) 1085–1096.
[57] K.E. Wilk, S.J. Harrop, L. Jankova, D. Edler, G. Keenan, F. Sharples, R.G. Hiller, P.M.G. Curmi, Evolution of a light-harvesting protein by addition of new subunits and rearrangement of conserved elements: crystal structure of a cryptophyte phycoerythrin at 1.63-Å resolution, Proc. Natl. Acad. Sci. U. S. A. 96 (1999) 8901–8906.
[58] J.G. Cobley, A.C. Clark, S. Weerasurya, F.A. Queseda, J.Y. Xiao, N. Bandrapali, I. D'Silva, M. Thounaojam, J.F. Oda, T. Sumiyoshi, M.H. Chu, CpeR is an activator required for expression of the phycoerythrin operon (cpeBA) in the cyanobacterium *Fremyella diplosiphon* and is encoded in the phycoerythrin linker–polypeptide operon (cpeC-DESTR), Mol. Microbiol. 44 (2002) 1517–1531.
[59] P. Maxson, K. Sauer, J. Zhou, D.A. Bryant, A.N. Glazer, Spectroscopic studies of cyanobacterial phycobilsomes lacking core polypeptides, Biochim. Biophys. Acta 977 (1989) 40–51.
[60] A.R. Grossman, M.R. Schaefer, G.G. Chiang, J.L. Collier, The phycobilisome, a light-harvesting complex responsive to environmental conditions, Microbiol. Rev. 57 (1993) 725–749.
[61] D. Mazel, P. Marlière, Adaptive eradication of methionine and cysteine from cyanobacterial light-harvesting proteins, Nature 341 (1989) 248.
[62] R. de Lorimier, G. Guglielmi, D.A. Bryant, S.E. Stevens Jr., Structure and mutation of a gene encoding a Mr 33,000 phycocyanin-associated linker polypeptide, Arch. Microbiol. 153 (1990) 541–549.
[63] R.E. Glick, B.A. Zilinskas, Role of the colorless polypeptides in phycobilisome. Reconstitution from separated phycobiliproteins, Plant Physiol. 69 (1982) 991–997.
[64] P. Fuglistaller, M. Mimuro, F. Suter, H. Zuber, Allophycocyanin complexes of the phycobilisome from *Mastigocladus laminosus*. Influence of the linker polypeptide $L_C^{8.9}$ on the spectral properties of the phycobiliprotein subunits, Biol. Chem. Hoppe-Seyler 368 (1987) 353–367.
[65] Y.Z. Zhang, X.L. Chen, L.S. Wang, B.C. Zhou, J.A. He, D.X. Shi, S.J. Pang, In vitro assembly of R-phycoerythrin from marine red alga *Polysiphonia urceolata*, Acta Biochim. Biophys. Sin. 34 (2002) 99–103.
[66] D.M. Kehoe, A.R. Grossman, Similarity of a chromatic adaption sensor to phytochrome and ethylene receptors, Science 273 (1996) 1409–1412.
[67] A.R. Grossman, A molecular understanding of complementary chromatic adaptation, Photosynth. Res. 76 (2003) 207–215.
[68] A.R. Grossman, D.M. Kehoe, Phosphorelay control of phycobilisome biogenesis during complementary chromatic adaptation, Photosynth. Res. 53 (1997) 95–108.
[69] F. Garnier, J.P. Dubacq, J.C. Thomas, Evidence for a transient association of new proteins with the *Spirulina maxima* phycobilisome in relation to light intensity, Plant Physiol. 106 (1994) 747–754.
[70] S. Schneider, C.J. Prenzel, G. Brehm, L. Gottschalk, K.H. Zhao, H. Scheer, Resonance-enhanced CARS spectroscopy of biliproteins: influence of aggregation and linker proteins on chromophore structure in allophycocyanin (*Mastigocladus laminosus*), Photochem. Photobiol. 62 (1995) 847–854.
[71] L. Gottschalk, F. Lottspeich, H. Scheer, Reconstitution of an allophycocyanin trimer complex containing the C-terminal 21–23 kDa domain of the core–membrane linker polypeptide Lcm, Z. Naturforsch., C 49 (1994) 331–336.
[72] K.E. Apt, N.E. Hoffman, A.R. Grossman, The gamma subunit of R-phycoerythrin and its possible mode of transport into the plastid of red algae, J. Biol. Chem. 268 (1993) 16208–16215.
[73] N.T. Marsac, Phycobiliproteins and phycobilisomes: the early observations, Photosynth. Res. 76 (2003) 197–205.
[74] L.N. Liu, X.L. Chen, X.Y. Zhang, Y.Z. Zhang, B.C. Zhou, One-step chromatography method for efficient separation and purification of R-phycoerythrin from *Polysiphonia urceolata*, J. Biotechnol. 116 (2005) 91–100.

[75] Z.Z. Pan, B.C. Zhou, C.K. Tseng, Comparative studies on spectral properties of R-phycoerythrin from the red seaweeds from Qingdao, Chin. J. Oceanol. Limnol. 4 (1986) 353–359.

[76] S. Ritter, R.G. Hiller, P.M. Wrench, W. Welte, K. Diederichs, Crystal structure of a phycourobilin-containing phycoerythrin at 1.90-Å resolution, J. Struct. Biol. 126 (1999) 86–97.

[77] K.E. Apt, S. Metzner, A.R. Grossman, The γ subunits of phycoerythrin from a red alga: position in phycobilisomes and sequence characterization, J. Phycol. 37 (2001) 64–70.

[78] S.M. Wilbanks, A.N. Glazer, Rod structure of a phycoerythrin II-containing phycobilisome: II. Complete sequence and bilin attachment site of a phycoerythrin gamma subunit, J. Biol. Chem. 268 (1993) 1236–1241.

[79] C. Chen, Y.Z. Zhang, X.L. Chen, B.C. Zhou, P.J. Gao, Langmuir–Blodgett film of phycobilisomes from blue-green alga *Spirulina platensis*, Acta Biochim. Biophys. Sin. 35 (2003) 952–955.

[80] K.H. Zhao, P. Su, S. Bohm, B. Song, M. Zhou, C. Bubenzer, H. Scheer, Reconstitution of phycobilisome core–membrane linker, L_{CM}, by autocatalytic chromophore binding to ApcE, Biochim. Biophys. Acta 1706 (2005) 81–87.